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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300, OECD 426

PC CODE: 047501 **DP BARCODE:** D405615

TEST MATERIAL (PURITY): Isopropanol (99% a.i.)

SYNONYMS: Isopropyl alcohol; 2-propanol

CITATION: Bates, H. and F. de Serres, (1991). Developmental neurotoxicity evaluation of isopropanol (CAS 67-63-0) administered by gavage to time-mated CD® rats on gestation day 6 through postnatal day 21. Research Triangle Institute (Research Triangle Park, NC). RTI Number 311C-4557, August 15, 1991. MRID 46895710. Unpublished.

SPONSOR: American Chemistry Council; Arlington, VA 22209

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46895710) isopropanol (99.9 a.i., batch/lot 6361-02-01) was administered to 64 female Sprague Dawley rats per dose by gavage at dose levels of 0, 200, 700, or 1200 mg/kg bw/day from gestation day (gd) 6 through postnatal day (PND) 21.

On day 4 postpartum, litter size was culled by standardized randomization to either a 4:4: or 5:3 sex ratio after pups were examined. After the litters were culled, one male and one female were assigned to each behavior test. Behavior tests were comprised of a motor activity test, an auditory startle response, and an active avoidance test (i.e., a learning and memory test). The fourth male and female or the single pup (in the case of a 5:3 sex ratio) was sacrificed on PND 22 and subjected to a neuropathological assessment and/or measurement of brain weight. For the behavior tests, two animals from each dose group were tested in each session when possible, with males tested first, followed be the females. Pups from each dose group were then rotated through the test chambers so that no single chamber was used to test all animals from a dose group. On PND 22 and 68, one male and one female pup from each litter were sacrificed. At each sacrifice, 24 pups were perfused *in situ* and examined for possible histopathologic lesions of the central and peripheral nervous system.

One high-dose dam died on PND 15. There were no other treatment-related signs of toxicity observed at any dose group. **The maternal LOAEL is 1200 mg/kg/day, based on mortality. The maternal NOAEL is 700 mg/kg/day.**

There were no treatment-related signs of toxicity in offspring at any dose level. Additionally, there were no treatment-related changes in behavior testing and/or neuropathological testing

when treated animals were compared with the controls. **The offspring NOAEL is ≥1200 mg/kg/day. The NOAEL for developmental neurotoxicity is ≥1200 mg/kg/day. The LOAEL could not be calculated.**

The major deficiency of this study is that the study investigators did not adequately explain the variations in numbers of pups evaluated for different parameters and at different time intervals. Parameters where the variation in the number of pups is a concern are generally the behavioral tests and brain weight data. For example, the number of female animals used to calculate the mean percent avoidances and the mean percent escapes differed by endpoint (i.e., the number used to calculate mean percent avoidances differed from that used to calculate the mean percent escapes), as well as session (i.e., the number used to calculate mean percent avoidances differed from session 1 to session 2). The study investigators failed to note why this was done. Without an explanation, the possibility of excluding animals exhibiting treatment-related effects cannot be eliminated. In the protocol deviations, study investigators did provide some of this information, such as stating that pup number 102-5 was inadvertently not test for motor skill activity on PND 13, but for the most part this type of explanation was not provided. Additionally, positive and historical control data were not provided.

This study is classified **ACCEPTABLE / Not Guideline** and does not satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, OECD 426).

COMPLIANCE: Signed and dated GLP and Quality Assurance statements are provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Isopropanol

Description:	Colorless liquid
Lot/batch #:	6361-02-01
Purity:	99.95±0.1% a.i.
Compound stability:	Not reported
CAS #of TGAI:	67-63-0
Structure:[1]	

2. Vehicle and/or positive control: Deionized/distilled water; lot/batch # and purity not provided

3. Test animals:

Species:	Rat (pregnant females)	
Strain:	Caesarean-originated, Virus Antibody Free (VAF) CD®(SD)BR outbred albino rats	
Age/weight at study initiation:	~9 weeks of age; 174-197 g	
Source:	Charles River Laboratories, Inc. (Raleigh, NC)	
Housing:	Dams were individually-housed in solid bottom polycarbonate cages with stainless steel wire lids.	
Diet:	Purina Certified Rodent Chow® (#5002) was provided *ad libitum*. The diet did not contain levels of contaminants that would interfere with the study.	
Water:	Deionized/filtered tap water from the Durham, North Carolina water system was available *ad libitum*. Water did not contain levels of contaminants that would interfere with the study.	
Environmental conditions:	**Temperature:**	20-24EC was the target temperature (mean was ~22.2° ±0.4° C)
	Humidity:	40-70% was the target humidity (mean was 55.8±2.6%)
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Animals were observed for 6 days prior to dosing	

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start: July 11, 1990; End: December 11, 1990

2. Study schedule: Mated dams were assigned to the study. The test substance was administered to the maternal animals from gestation day (gd) 6 through postnatal day (PND) 21. Pups were weaned on PND 21, and maternal animals were sacrificed on PND 22. Pups remained on study until either PND 22 or 68.

[1] Source: www.chemfinder.com

3. **Mating procedure:** Females were pregnant on arrival. At Charles River Laboratories, Inc., females were paired with males until evidence of mating was observed (i.e., copulatory plug). The day on which evidence of mating was observed was designated as gd 0.

4. **Animal assignment:** Dams were assigned to the dose groups indicated in Table 1 by stratified randomization methods designed to provide uniform mean body weights across dose groups.

   Offspring were assigned to testing subgroups at the time of litter standardization on PND 4 (Table 1). On PND 4, litter size was culled by standardized randomization to either a 4:4: or 5:3 sex ratio after pups were examined. After the litters were culled, one male and one female were assigned to each behavior test. The fourth male and female or the single pup (in the case of a 5:3 sex ratio) was sacrificed on PND 22 and subjected to a neuropathological assessment and/or measurement of brain weight. For the behavior tests, two animals from each dose group were tested in each session when possible, with males tested first, followed be the females. Pups from each dose group were then rotated through the test chambers so that no single chamber was used to test all animals from a dose group. On PND 22 and 68, one male and one female pup from each litter were sacrificed. At each sacrifice, 24 pups were perfused *in situ* and examined for possible histopathologic lesions of the central and peripheral nervous system.

TABLE 1. Study Design

Experimental parameter	Dose (mg/kg/day)			
	0	200	700	1200
Maternal animals				
No. of maternal animals assigned	64	64	64	64
Offspring				
Detailed clinical observation	All	All	All	All
Motor activity (PND 13, 17, 21, 47, 58)	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Auditory startle habituation (PND 22 and 60)	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Active avoidance testing (PND 60-64)	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Brain weight				
PND 22	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
PND 68	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Neuropathology				
PND 22	3/sex	3/sex	3/sex	3/sex
PND 68	3/sex	3/sex	3/sex	3/sex

5. **Dose selection rationale:** The dose levels were selected based on the results from the following four studies: 1) a preliminary study for a multi-generation rat reproduction study conducted at Exxon Biomedical Sciences, Inc.; 2) a preliminary toxicity evaluation for a teratology study; 3) a definitive teratology study; and 4) the preliminary evaluation for this developmental neurotoxicity study. All studies used CD® rats exposed to isopropanol via gavage under similar conditions.

   For the preliminary multi-generation rat reproduction study conducted at Exxon Biomedical Sciences, Inc., 10 rats/sex/group received 1, 100, 500, 1750, or 2500 mg/kg/day of isopropanol for 10 weeks prior to and during mating. Females continued to be treated after mating until sacrifice on PND 21. At the highest dose, animals revealed some clinical signs

of toxicity immediately after dosing, and all animals died or were sacrificed moribund prior to mating. The incidence of mortality in the control, 100-, 500-, 1000-, and 1750-mg/kg/day groups was 0/20, 0/20, 1/20, 2/20 and 5/20, respectively. Significant decreases in food consumption during mating were observed in 1750-mg/kg/day females. Additionally, there were small decreases in mean body weight for all groups during gestation when compared to the control. All 1750-mg/kg/day pups died by PND 5.

For the preliminary teratology study, 12 dams/group were administered 0, 625, 1250, or 2500 mg/kg/day via gavage from gd 5 through 15. Exposure to the high dose resulted in 11/11 deaths; seven of these deaths occurred before gd 10 and four occurred on either gd 12 or 13. An additional 2/12 dams treated with 1250 mg/kg/day also died prior to scheduled necropsy. Additionally, significant reductions in maternal body weight gain, decreased food consumption, and clinical signs of toxicity were observed in mid- and high-dose dams.

For the definitive teratology study, 25 dams /group were administered 0, 400, 800, or 1200 mg/kg/day of isopropanol from gd 6 through 15. Mortality was observed in 1/25 mid-dose dams and 2/25 high-dose dams. There were few other signs of maternal toxicity. Significant fetal body weight reductions were observed at 800 and 1200 mg/kg/day.

For the preliminary developmental neurotoxicity study, 10 dams/group were exposed to 0, 200, 400, 800, or 1200 mg/kg/day from gd 6 through PND 21. At the highest dose, minimal clinical signs of toxicity were observed. Maternal weight parameters and food consumption showed no significant alterations; however, the highest exposure group exhibited a 6-10% reduction in mean maternal weight gain throughout the study.

Consequently, the high dose was based on PEA TSCA guideline CFR 795.250, which specifies that the high exposure level must induce some maternal toxicity but not enough to result in a maternal weight gain reduction of more than 20% when compared to controls. Additionally, lethality data was used when determining the high dose. The occurrence of mortality in the four studies was previously discussed. For the preliminary developmental neurotoxicity study, the LD_{15} was determined to be 1200 mg/kg/day. Since maternal body weight gain was not decreased by more than 20% from the control at 1200 mg/kg/day for the preliminary developmental neurotoxicity study and minimal maternal mortality is expected at 1200 mg/kg/day, all requirements of the guidelines were expected to be met with a high dose of 1200 mg/kg/day.

Additionally, the guideline requires a low dose, i.e., the maternal/pup NOAEL, and a mid-dose, i.e., the dose halfway between the low and high dose. In the four studies evaluated, a NOAEL of 400-600 mg/kg/day form gd 6-15 was established. However, developmental neurotoxicity studies require that animals be exposed from gd 6 to PND 21. Because of the protracted dosing regimen, a slightly lower NOAEL was considered necessary. Consequently, the investigators selected a low dose of 200 mg/kg/day and a mid dose of 700 mg/kg/day.

6. **Dosage administration:** All doses were administered once daily by gavage, on gd 6 through PND 21, in a volume of 5 mL/kg of body weight/day. Dosing was based on the body weight from the most recent body weight determination.

7. **Dosage preparation and analysis:** Test material-vehicle mixture was prepared in sufficient quantity for the period of dosing by dissolving appropriate amounts of test substance with distilled/deionized water. Concentrations were determined by dividing the dose level (mg/kg/day) by the dose volume (5 mL/kg). Prior to the start of the study, formulations of the test substance in distilled/deionized water were evaluated for homogeneity, stability, and achieved concentration. Samples (0.100 mL) of test formulations from all four dose groups (i.e., control, 200, 700, and 1200 mg/kg/day) were taken in triplicate and analyzed by gas chromatography.

Results:

Homogeneity analysis: Test formulations were found to be homogenous, with the coefficient of variation ranging from 0.2 to 2.2.

Stability analysis: Test formulations were stable for at least 49 days under refrigerated conditions.

Concentration analysis: Test formulations assayed were 94.3-103% of the nominal concentration.

The analytical data indicate that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS:

1. In-life observations:

a. Maternal animals: All dams were observed for clinical signs of toxicity (including neurotoxicity) at least once daily on gd 0-5 (prior to dosing) and at least twice daily (once at dosing and again 1-2 hours after dosing) during the dosing period (gd 6 through PND 21). Additional unscheduled observations made immediately after dosing also were recorded. The study report and protocol do not specify whether any observations were made outside the home cage (guidelines recommend that ten dams per dose group be observed outside the home cage at least twice during gestation and twice during lacation). According to the study protocol, observations were made by a technician blinded to dose conditions for:

1. Any response with respect to body position, activity, coordination, or gait.
2. Any unusual behavior such as head flicking, compulsive biting or licking, circling, etc.
3. The presence of:
   - Convulsions or tremors
   - Increased salivation
   - Increased lacrimation or red colored tears
   - Increased/decreased urination or defecation (including diarrhea)
   - Piloerection
   - Mydriasis or miosis (enlarged or constricted pupils)
   - Unusual respiration (fast, slow, gasping, or retching)
   - Vocalization

Body weight data were recorded on gd 0, 6, 9, 12, 15, 18, and 20 and on PND 0, 4, 7, 13, 17, and 21. Food consumption data were measured for the intervals gd 0-6, 6-9, 9-12, 12-15, 15-18, and 18-20, as well as PND 0-3, 3-6, and 9-12.

b. Offspring:

1. **Litter observations:** The day of completion of parturition was designated as PND 0. Pups were observed for clinical signs of toxicity once daily, beginning on PND 0. Pups were counted, examined externally, weighed, and sexed on PND 0 and 4.

   On PND 4, litter size was culled by standardized randomization to either a 4:4 or 5:3 sex ratio after pups were examined. Litters with an insufficient number of pups were removed from the study after culling, while those with a sufficient number of pups remained. Pups from the remaining litters were examined and weighed on PND 7, 13, 17, and 21.

2. **Developmental landmarks:** Testicular descent (males) and vaginal opening (females) were monitored daily starting at PND 20 or 30, respectively. Other developmental landmarks (e.g., eye opening, pinna unfolding, and incisor eruption) were not monitored or evaluated in the study.

3. **Postweaning observations:** After weaning on PND 21, offspring were observed for clinical signs of toxicity once daily until termination. Individual offspring body weight data were recorded on PND 36, 49, and 68.

4. **Neurobehavioral evaluations:** The pups from each litter were randomly distributed into 4 male/female pairs. One male/female pair from each litter was assigned to each of three behavioral tests: motor activity, auditory startle reflex, and active avoidance. (The remaining pair was sacrificed on PND 22, see Section C.2.b). Observations from the behavioral tests and the schedule for those observations are summarized as follows from the report.

   i. **Functional observational battery (FOB):** A FOB assessment was not conducted on pups.

   ii. **Motor activity testing:** Motor activity was conducted in a figure-8 maze on PND 13, 17, 21, 47, and 58. Testing lasted for one continuous hour, with activity count subtotaling twelve 5-minute segments. Prior to the testing, the motor activity apparatus was set up to collect data that was stored in a calibration file. All eight figure-8 mazes used in the session operated without interruption, recording that no photocell beams were broken during the session. Photocell beams were then manually broken and recorded as fully operational after the program finished recording that no photocell beams were broken during the session. This procedure was conducted before and after every session on each day of testing.

   iii. **Auditory startle reflex habituation:** Auditory startle reflex was assessed on PND 22 and 60. Animals were acclimatized for 5 minutes before an ~120 db tone was sounded for 50 milliseconds. A computer system monitored the animal's response

during the tone and for 50 milliseconds after the tone stopped and then paused for 8 seconds. The procedure was repeated 50 times per session (i.e., five contiguous 10 trial blocks). The maximum amplitude of each startle response and the latency time for the maximum response was recorded. Prior to testing, the auditory startle apparatus was set up to collect data that was stored in a calibration file. Each of the eight startle boxes was calibrated to respond equally to a device that vibrated at a preset frequency and amplitude. Another file, created prior to and after each session, recorded responses to taps on each of the eight sensing devices and documented that the devices were operational before and after each session.

iv. **Learning and memory testing:** Active avoidance testing was assessed for 5 consecutive days on PND 60-64. Animals were acclimatized to the test apparatus for 5 minutes before activating the conditioning stimulus, which consisted of a continuous light (6 watts) and sound (~85 db) that lasted for 10 seconds followed by a mild electric current (~0.9 milliamp) for a maximum of 30 seconds. The light and sound were presented on the side of the shuttle box on which the animal stood, and the mild electric current was delivered at the foot grid. The intertrial interval time was 30±15 seconds. A successful trial was terminated when the animal shuttled to the opposite side of the apparatus after the warning stimulus to avoid the shock. Twenty trials (2 blocks of 10 contiguous trials) were conducted daily. The following responses were recorded: the number of adaptation period crossings, intertrial crossings, avoidances and escapes, time to avoidance, and time to escape (shock time). The shock, lights, and sound of each shuttle box were checked prior to each session. Shock was set at ~0.9 milliamps prior to and after each session. The test apparatus was checked to make sure that shuttles could be determined by manually breaking the photocell beam in each box to simulate the movement of an animal.

2. **Postmortem observations:**

a. **Maternal animals:** Dams that survived until scheduled necropsy were sacrificed on PND 22 via carbon dioxide asphyxiation. Dams were then evaluated for terminal body weight, liver and kidney weight, and uterine implants. Dams that did not survive until scheduled necropsy or that were sacrificed after culling on PND 4 were subjected to a necropsy, but their tissues were not weighed or saved.

b. **Offspring**: On PND 22 and 68, one male and one female pup from each litter was sacrificed via carbon dioxide asphyxiation and weighed. At each sacrifice, 24 pups were perfused *in situ* with 4% paraformaldehyde in 0.1M phosphate buffer and examined for possible histopathologic lesions of the central and peripheral nervous system. The specific tissues examined are shown in the following Table 2. These tissues were embedded in paraffin (except for the sural nerve which was embedded in GMA), sectioned, and stained with hematoxylin and eosin.

Table 2. Tissues Examined

The CHECKED (X) tissues from the pups were microscopically evaluated.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain	X	Mid-thigh
X	Center of cerebrum	X	Sciatic notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural nerve
X	Medulla oblongata	X	Tibial nerve
	SPINAL CORD		Peroneal nerve
X	Cervical swelling		Lumbar dorsal root fibers
X	Lumbar swelling		Lumbar dorsal root ganglion
	OTHER		Lumbar ventral root fibers
X	Gasserian ganglion		Cervical dorsal root ganglion
	Trigeminal nerves		Cervical dorsal root fibers
	Optic nerve		Cervical ventral root fibers
	Eyes	X	Dorsal root ganglia
		X	Dorsal root fibers
		X	Ventral root fibers

From the remaining animals, brains were removed, separated into the telencephalon, diencephalon, medulla oblongata/pons, and the cerebellum, and weighed. Pups not assigned for brain weights or for histopathological examination on PND 68 were removed from the study and sacrificed. Detailed morphometric evaluation of the neocortex, hippocampus, and cerebellum was not conducted.

D. DATA ANALYSIS:

1. **Statistical analyses:** The parameters evaluated and the methods used to determine their statistical significance are summarized below. Appropriate adjustments for multiple comparisons were applied to all p-values in order to maintain overall alpha type I error rates and reduce the likelihood of obtaining spurious results. P-values obtained from a series of analyses conducted within a single endpoint were adjusted for multiple comparisons of treated groups to control. Further p-value adjustments were then applied to maintain the type I error rate for sets of related endpoints. Both the unadjusted and adjusted p-values were reported.

   The variable Habituated/Exploratory Ratio, which is the mean motor activity of the animals for the last 5 minutes of the session divided by the mean motor activity during the first 5 minutes of the session, was originally considered as an endpoint for this study. Data showed that this ratio magnifies minor differences in the final and initial motor activity without accounting for the activity profile of time. Consequently, findings regarding this ratio were discounted and were not discussed further in the study report.

   Table 3. The Parameters Evaluated and the Methods used to Determine the Statistical Significance

Parameter(s)	Statistical Method
Discrete variables (i.e., those indicating presence/absence of adverse effects, such as pup death indices)	Contingency table techniques consisting of Pearson's chi-square test for overall heterogeneity followed be individual exposed vs. control group comparisons using Fisher's Exact test (2-sided) and the Cochran-Armitage chi-square test for trend (2-sided)
Continuous variables (e.g., body weights, food consumption, organ weights) and incidence data (e.g., number live pups on PND 0)	Parametric or non-parametric tests (i.e., if assumptions for parametric tests were not satisfied, non-parametric analyses were employed). Tests used to make this determination consisted of Bartlett's test for homogeneity of variance ($p<0.01$) or Shapiro-Wilks' test for normality ($p<0.05$). Parametric analyses included Dunnett's t-test for simultaneous exposed vs. control group comparisons ($p<0.05$, <0.01, and <0.001; 2-sided), followed by a linear contrast for monotonic trend (2-sided F-test). Dunnett's test adjusts the overall type 1 error rate for multiple comparisons, eliminating the need to perform a preliminary test for overall heterogeneity. Non-parametric analyses include Fligner's test for simultaneous exposed vs. control group comparisons ($p<0.05$, <0.01, and <0.001), followed by Jonckheere's test for trend (constructed as two 1 sided tests). The overall Kruskal-Wallis test for heterogeneity was not considered before comparing the exposed group to the control.
Continuous variable in which the experimental unit is the dam, but the focus is on the individual pup as the observational unit (e.g., time to vaginal opening and testis descent)	Nested analysis of variance to account for intra-litter correlation Statistical analyses included Dunnett's t-test for simultaneous exposed vs. control group comparisons ($p<0.05$, <0.01, and <0.001; 2-sided), followed by linear contrast for monotonic trend (2-sided F-test)
Behavioral variables (e.g., 1-hour activity in motor activity trials; the percentage avoidance trials, the number of adaptation crossings, and the mean number of inter-trial interval crossing in active avoidance sessions; and the maximum startle amplitude and time to maximum amplitude in auditory startle trials)	Analysis of variance with repeated measures on the experimental unit (i.e., the pup) over time (e.g., day, session, block) constructed separately for each sex
Variables that measure time to some event (e.g., habituation onset time in motor activity trials and avoidance/escape time in active avoidance sessions)	Survival analysis techniques

2. Indices:

a. Reproductive indices: No reproductive indices were presented or evaluated in the study report.

b. **Offspring viability indices:** No offspring viability indices were presented or evaluated in the study report.

3. **Positive and historical control data:** Positive and historical control data were not provided.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** One high-dose dam died on PND 15. There were no treatment-related clinical observations made for any of the treated dams.

2. **Body weight and food consumption:** Selected group mean body weights and food consumption values for pregnant or nursing dams are summarized in Table 4 below. There were no treatment-related changes in body weight or body weight gain for any of the treated dams.

   There were no treatment-related changes in food consumption for any of the treated dams. One mid-dose dam exhibited a significant increase in food consumption during PND 0-3; however, this observation was incidental and was not considered treatment-related.

TABLE 4. Mean (±Standard Deviation) maternal body weight and food consumption [a]

Observations/study week	Dose (mg/kg/day)			
	Control	200	700	1200
Gestation				
Mean body weight (g)				
gd 0	184.65±1.07	185.86±0.89	187.73±0.80	186.03±1.02
N	34	35	31	35
gd 6	230.45±1.73	232.41±1.50	234.65±1.62	234.06±1.69
N	34	35	31	35
gd 9	251.08±2.03	251.70±1.83	253.94±2.00	250.93±1.54
N	34	35	31	35
gd 12	271.71±2.53	274.30±2.18	278.25±2.32	274.48±2.18
N	34	35	31	35
gd 15	297.54±3.04	299.93±2.62	301.41±3.07	297.57±2.68
N	34	35	31	35
gd 18	330.71±4.29	333.11±3.69	355.00±4.09	331.84±3.85
N	34	35	31	35
gd 20	358.49±5.20	363.11±4.42	363.87±4.68	359.06±4.32
N	34	35	31	35

TABLE 4. Mean (±Standard Deviation) maternal body weight and food consumption [a]

Observations/study week	Dose (mg/kg/day)			
	Control	200	700	1200
Mean weight gain (g)				
gd 6-20	128.04±4.30	130.70±3.73	129.21±4.03	125.00±3.63
N	34	35	31	35
Mean food consumption (g/animal/day)				
gd 0-6	108.44±1.76	109.42±1.90	109.52±1.81	112.51±1.81
N	34	35	31	35
gd 6-9	68.49±1.19	68.54±0.977	69.11±1.62	65.644±1.27
N	34	35	31	35
gd 9-12	70.82±2.25	74.07±1.68	74.60±1.34	74.16±1.41
N	34	35	31	35
gd 12-15	77.76±1.37	78.50±1.27	79.06±1.47	79.83±1.31
N	34	35	31	35
gd 15-18	86.00±1.64	88.76±1.52	87.27±1.48	88.26±1.46
N	34	35	31	35
gd 18-20	54.04±1.09	55.58±0.94	55.51±1.18	54.61±1.23
N	34	35	31	35
Postnatal				
Mean body weight (g)				
PND 0	288.70±3.02	291.45±2.63	286.50±3.581	285.67±3.32
N	33	35	30	34
PND 4	302.87±3.10	306.83±3.56	309.39±4.17	299.66±3.53
N	33	35	30	34
PND 7	311.94±3.34	314.80±3.562	317.80±4.81	306.94±3.32
N	27	27	26	29
PND 13	328.33±4.75	333.34±4.19	334.64±4.84	327.24±3.63
N	27	27	26	29
PND 17	341.92±3.64	343.72±4.16	342.00±5.20	339.11±3.68
N	27	27	26	29
PND 21	329.53±3.74	329.45±3.94	333.44±4.22	329.96±3.31
N	27	27	26	29
Mean weight gain (g)				
PND 0-21	39.64±2.96	38.04±2.85	46.30±2.98	43.93±2.83
N	27	27	26	29
Mean food consumption (g/animal/day)				
PND 0-3	86.54±3.06	92.44±4.44	101.53±4.52	93.51±3.25
N	33	35	30	34
PND 3-6	129.09±2.62	131.78±3.46	131.90±3.27	127.15±3.05
N	27	27	26	29
PND 6-9	159.58±4.56	160.68±3.73	159.61±3.12	153.767±2.54
N	27	27	26	29
PND 9-12	185.08±3.05	183.63±4.22	180.59±3.43	177.30±2.95
N	27	27	26	29

Data obtained from pages 281-284 in the study report.
N = Number of dams.

3. **Test substance intake:** Test formulations were administered via gavage. Consequently, the calculation of test substance intake was not needed, and animals received 0, 200, 700, or 1200 mg/kg/day.

4. **Reproductive performance:** Results for the maternal animals are summarized from the report in Table 5 below. There were no treatment-related changes in any of the endpoints measured.

TABLE 3. Reproductive performance [a]				
Observation	Dose (mg/kg/day)			
	0	200	700	1200
Number mated	64	64	64	64
Number pregnant	34	35	31	35
Number of litters	34	35	31	35
Intercurrent deaths	0	0	0	1 [b]
Number of usable litters [c]	27	27	26	31
Mean (±standard deviation) gestation duration (days)	21.6061±0.09672	21.5000±0.10555	21.7333±0.09509	21.8235±0.07865
Incidence of dystocia	NA	NA	NA	NA

[a] Data obtained from pages 29 and 285 in the study report.

[b] Because the dam died on PND 15, the pups did not receive the full 21 days of postnatal exposure; therefore, this litter was removed from the study.

[c] Number of litters tat could be culled to either a 4:4 or 5:3 sex ratio on PND 4.

NA = Not available

5. **Maternal postmortem results:** There were no treatment-related maternal postmortem findings.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability results from pups during lactation are summarized from the report in Table 6 below. There was a significant decrease in the number of deaths occurring on PND 0-4 at 700 mg/kg/day in males. This decrease was not considered biologically significant because it only occurred at the mid-dose level.

TABLE 6. Litter size and viability [a]

Observation	Dose (mg/kg/day)			
	0	200	700	1200
Total number born	NA	NA	NA	NA
Number born live	375	380	355	378
Number born dead	NA	NA	NA	NA
Sex Ratio PND 0	48.8±3.42	49.5±3.22	51.14±2.43	46.90±3.19
# Deaths PND 0-4	0.24±0.08	0.13±0.06	0.03±0.03*	0.43±0.10
% Survival	97.71±0.74	98.80±1.54	99.75±0.26	96.23±3.46
# Deaths PND 4-21	0.04±0.04	0.15±0.07	0.00±0.00	0.03±0.03
% Survival	99.54±0.43	98.15±0.87	100.00±0.00	99.57±0.43
Mean litter size:				
PND 0	11.48±0.54	11.03±0.52	11.87±0.59	11.38±0.55
PND 4 [b]	11.21±0.53	11.17±0.47	12.24±0.43	11.25±0.57
PND 4 [c]	NA	NA	NA	NA
PND 11	NA	NA	NA	NA
PND 17	NA	NA	NA	NA
PND 21	NA	NA	NA	NA
Live birth index	NA	NA	NA	NA
Viability index	NA	NA	NA	NA
Lactation index	NA	NA	NA	NA

[a] Data obtained from pages 31 and 367, 372, 377, 383, 388, 393 in the study report; means and standard error rates are presented as appropriate.

[b] Before standardization (culling).

[c] After standardization (culling).

NA = Not available

* Statistically different from control, $p<0.05$

2. **Body weight:** There were no treatment-related changes in offspring body weights during lactation. Selected mean preweaning pup body weight data are presented in the following tablet.

TABLE 7. Mean (±standard deviation) pre-weaning pup body weights (g) [a]

Postnatal day	Dose (mg/kg/day)							
	0	200	700	1200	0	200	700	1200
	Males				Females			
1	6.68±0.11	6.53±0.09	6.60±0.10	6.46±0.08	6.25±0.96	6.25±0.08	6.14±.0.10	6.07±0.10
4 [b]	10.61±0.25	10.81±0.22	10.62±0.24	10.38±0.18	10.02±0.22	10. 36±0.20	9.98±0.21	9.73±0.23
4 [c]	10.45±0.26	10.51±0.22	10.43±0.21	10.27±0.16	9.84±0.24	10.12±0.22	9.82±0.19	9.79±0.14
7	16.4±0.33	16.71±0.36	16.57±0.30	15.89±0.23	15.38±0.32	16.02±0.31	15.6±0.24	15.10±0.22
13	31.00±0.48	31.83±0.65	31.32±0.48	29.67±0.41	29.2±0.48	30.68±0.58	30.05±0.43	28.24±0.38
17	40.52±0.62	41.75±0.77	41.12±0.61	38.82±0.54	38.76±0.60	40.37±0.69	39.44±0.482	37.41±0.48
21	53.34±1.08	54.33±1.16	54.48±0.89	52.40±0.76	50.52±1.02	52.23±1.04	51.99±0.72	49.88±0.73

[a] Data obtained from pages 289-290 in the study report.
[b] Before standardization (culling).
[c] After standardization (culling).

There were no treatment-related changes in offspring postweaning body weights. Selected mean postweaning offspring body weight data are presented in Table 8 below.

TABLE 8. Mean (±standard deviation) post-weaning pup body weights (g) [a]

Postnatal day	Dose (mg/kg/day)							
	0	200	700	1200	0	200	700	1200
	Males				Females			
36	167.81±2.60768	169.23±2.88	171.18±2.67	167.58±2.16	142.66±1.97	144.16±2.04065	147.31±2.03	140.29±1.95
49	298.40±4.03461	300.00±4.08	299.09±3.61	295.07±3.39	21.63±2.199	211.03±2.31597	213.26±2.55	205.15±3.10
68	447.92±5.26790	449.28±5.78	443.53±5.65	432.55±4.40	267.89±3.22	270.82±2.86067	270.92±3.96	262.48±3.93

[a] Data obtained from pages 290 in the study report.

3. Developmental landmarks:

a. **Sexual maturation**: There were no treatment-related changes observed for the sexual maturation parameters examined. The data are presented in Table 9 below.

TABLE 9. Mean (±standard error rate) age of sexual maturation (days) [a]

Parameter	Dose (mg/kg/day)			
	0	200	700	1200
N (M/F)	81/81	81/81	78/78	86/87
Preputial separation (males)	21.21±0.15	21.33±0.16	21.01±0.16	20.95±0.11
Vaginal opening (females)	35.75±0.22	35.49±0.31	36.36±0.36	36.51±0.31

[a] Data obtained from pages 31 and 293 in the study report.

b. **Physical landmarks:** Physical landmarks were not presented or evaluated in the study report.

4. Behavioral assessments:

a. **Functional observational battery:** A FOB assessment was not conducted on pups.

b. **Motor activity:** Total activity data are presented in Table 10 below. There were no significant effects observed in any group at any observation compared to controls. There were no day-by-treatment interaction effects for either sex according to the repeated measures analysis. A steady increase in total 1-hour activity level was observed, reaching a maximum on PND 47 for all dose groups including the control group. All dose groups, including the control, exhibited a decrease in 1-hour activity level on PND 58 when compared to PND 47.

TABLE 10. Mean (±standard diviation) motor activity data (total activity counts for session) [a]

Test Day	Dose (mg/kg/day)			
	0	200	700	1200
Males				
PND 13	56.07±7.12 (27)	55.85±10.3318 (27)	57.38±9.01 (26)	69.27±8.28 (30)
PND 17	186.63±22.10 (27)	185.22±26.1290 (27)	202.15±25.66 (26)	217.17±21.78 (29)
PND 21	224.59±15.73 (27)	197.93±18.7884 (27)	210.77±14.67 (26)	217.11±15.13 (28)
PND 47	565.74±33.66 (27)	557.33±23.4183 (27)	528.35±35.95 (26)	550.68±28.28 (28)
PND 58	470.22±18.56 (27)	428.44±18.8659 (27)	431.85±28.58 (26)	445.14±24.38 (28)
Females				
PND 13	64.90±8.06 (27)	45.81±8.55 (26)	50.00±7.17 (26)	80.27±9.89 (30)
PND 17	208.30±25.73 (27)	150.37±21.26 (27)	218.23±20.36 (26)	230.52±27.33 (29)
PND 21	286.36±25.05 (25)	243.44±19.72 (25)	281.29±18.67 (24)	266.963±23.45 (27)
PND 47	715.26±39.83 (27)	700.33±31.66 (27)	712.69±29.851 (26)	714.24±31.30 (29)
PND 58	589.78±25.66 (27)	587.59±26.83 (27)	592.35±32.44 (26)	592.28±25.91 (29)

[a] Data obtained from pages 294 in the study report.
() The number of animals examined

c. **Auditory startle reflex habituation:** The amplitude and habituation data are presented in Tables 11a, 11b, and 11c below. There was a significant increase in maximum amplitude in 700-mg/kg/day males on PND 60 during block 2. Females treated with 200 mg/kg/day exhibited a significant, linear block-by-treatment interaction on PND 22 as evidenced by the steeper decline in startle amplitude across blocks when compared to the controls. This effect was not considered treatment-related, however, because only the low dose was affected and there was no dose-response relationship.

There were no treatment-related effects with regard to latency. On PND 22, there were no block-by-treatment interaction effects. On PND 60, 700-mg/kg/day males exhibited a significant quadratic block-by-treatment interaction effect, as evidenced by early reductions versus early increases in latency times for 700-mg/kg/day males when compared to the control group followed by relatively little change in latency times as the study progressed. This effect was not considered treatment related due to the relatively large variation of the latency means and the absence of a dose-response relationship.

TABLE 11a. Mean (±standard deviation) overall (blocks 1-5) acoustic startle peak amplitude (g) and latency to peak (MSEC)[a]

Dose (mg/kg/day)	Parameter	Males		Females	
		PND 22	PND 60	PND 22	PND 60
0	Peak Amp.	-26.86±3.65 (27)	-129.82±29.29 (27)	-27.16±3.67 (26)	-94.27±15.49 (27)
	Latency	-0.681±0.40 (27)	0.74±0.565 (27)	-0.82±0.38 (26)	-0.88±0.51 (27)
200	Peak Amp.	-29.28±4.40 (27)	-102.6±19.71 (27)	-38.75±4.55 (27)	-76.92±12.00 (27)
	Latency	-0.82±0.26672 (27)	0.49±0.51514 (27)	-0.05±0.33 (27)	0.105±0.46 (27)
700	Peak Amp.	-34.39±5.99 (26)	-110.38±25.1212 (26)	-23.13±3.05 (26)	-54.14±10.25 (26)
	Latency	-0.85±0.30 (26)	-1.07±0.54195 (26)	-0.51±0.420 (26)	0.37±0.37 (26)
1200	Peak Amp.	-33.28±4.33 (29)	-123.52±23.0528 (29)	-26.76±2.65 (29)	-119.21±24.73 (29)
	Latency	-1.24±0.36 (29)	-0.43±0.39613 (29)	-0.814±0.34 (29)	-0.81±0.39 (29)

[a] Data were obtained from pages 301-302.
() The number of animals examined

TABLE 11b. Mean (±standard deviation) interval acoustic startle peak amplitude (g) and latency to peak (MSEC) in F_1 male rats [a]

Dose (mg/kg/day)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 22						
0	Peak Amp.	237.60±28.04 (27)	152.58±22.93 (27)	155.42±26.39 (27)	134.63±17.29 (27)	130.17±16.70 (27)
	Latency	35.36±1.21 (27)	32.81±1.53 (27)	31.493±1.40 (27)	33.14±1.58 (27)	32.63±1.71 (27)
200	Peak Amp.	270.51±18.89 (27)	184.40±20.05 (27)	169.57±18.53 (27)	162.51±17.29 (27)	153.37±18.107 (27)
	Latency	34.19±1.40 (27)	31.62±1.60 (27)	30.03±1.09 (27)	29.88±0.92 (27)	30.93±0.98 (27)
700	Peak Amp.	271.60±24.63 (26)	184.33±13.45 (26)	155.073±12.50 (26)	140.12±12.12 (26)	134.06±11.54 (26)
	Latency	33.41±1.57 (26)	29.09±0.97 (26)	30.66±1.27 (26)	32.35±1.79 (26)	30.01±1.35 (26)
1200	Peak Amp.	286.23±25.52 (29)	201.43±19.16 (29)	163.93±16.45 (29)	159.95±17.33 (29)	153.11±16.30 (29)
	Latency	32.69±1.30 (29)	30.09±1.13 (29)	29.47±1.30 (29)	29.40±1.09 (29)	27.74±0.85 (29)
PND 60						
0	Peak Amp.	800.97±144.03 (27)	331.38±58.56 (27)	329.58±49.84 (27)	296.14±48.37 (27)	281.70±37.18 (27)
	Latency	39.50±1.78 (27)	43.91±2.78 (27)	43.58±2.58 (27)	43.29±2.36 (27)	42.46±2.77 (27)
200	Peak Amp.	822.25±117.62 (27)	519.26±115.74 (27)	464.92±138.58 (27)	433.67±117.65 (27)	411.91±115.99 (27)
	Latency	40.81±1.92 (27)	43.58±2.36 (27)	44.17±2.33 (27)	452.18±2.44 (27)	42.77±2.49 (27)
700	Peak Amp.	849.28±127.11 (26)	623.80±113.67 (26)	434.04±68.97 (26)	372.75±57.77 (26)	407.78±73.28 (26)
	Latency	43.10±2.15 (26)	38.03±1.80 (26)	39.76±2.64 (26)	38.61±2.33 (26)	38.82±2.29 (26)
1200	Peak Amp.	906.49±132.53 (29)	548.03±99.81 (29)	499.81±100.75 (29)	472.60±91.84 (29)	412.41±64.98 (29)
	Latency	41.36±1.59 (29)	42.83±2.08 (29)	43.53±2.67 (29)	43.93±2.44 (29)	39.63±2.19 (29)

[a] Data were obtained from pages 298 and 300; 10 trials/block.
() The number of animals examined

TABLE 11c. Mean (±standard deviation) interval acoustic startle peak amplitude (g) and latency to peak (MSEC) in F_1 female rats [a]

Dose (mg/kg/day)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 22						
0	Peak Amp.	247.56±20.46 (26)	172.39±14.66 (26)	144.36±12.20 (26)	140.83±11.90 (26)	138.93±12.69 (26)
	Latency	33.43±1.70 (26)	31.43±1.071 (26)	30.49±1.08 (26)	30.55±0.91 (26)	30.14±1.14 (26)
200	Peak Amp.	279.86±24.26 (27)	189.04±21.72 (27)	174.19±24.60 (27)	146.71±21.35 (27)	124.87±14.18 (27)
	Latency	31.11±0.92 (27)	31.99±1.52 (27)	31.67±0.98 (27)	30.56±0.90 (27)	30.93±0.94 (27)
700	Peak Amp.	241.85±16.69 (26)	179.61±14.63 (26)	148.55±11.55 (26)	136.86±10.80 (26)	149.33±13.27 (26)
	Latency	32.96±1.16 (26)	28.98±1.02 (26)	30.13±1.11 (26)	31.78±1.37 (26)	30.90±1.13 (26)
1200	Peak Amp.	261.87±18.07 (29)	186.85±15.94 (29)	165.52±13.68 (29)	170.79±16.06 (29)	154.84±14.46 (29)
	Latency	35.40±1.34 (29)	30.78±1.188 (29)	31.99±1.31 (29)	30.12±1.25 (29)	32.16±1.26 (29)
PND 60						
0	Peak Amp.	759.07±106.89 (27)	464.01±76.83 (27)	380.60±66.50 (27)	373.59±58.90 (27)	382.00±61.71 (27)
	Latency	39.04±1.59 (27)	37.98±1.60 (27)	36.66±2.27 (27)	34.77±1.60 (27)	35.50±1.87 (27)
200	Peak Amp.	667.71±123.05 (27)	447.98±106.15 (27)	329.59±69.03 (27)	322.54±60.74 (27)	360.04±87.04 (27)
	Latency	38.20±1.64 (27)	39.82±2.48 (27)	39.20±2.34 (27)	39.14±2.51 (27)	38.61±2.74 (27)
700	Peak Amp.	485.76±68.39 (26)	291.20±34.39 (26)	259.32±24.77 (26)	274.75±34.36 (26)	269.21±39.76 (26)
	Latency	38.28±1.79 (26)	38.60±2.17 (26)	38.92±2.01 (26)	37.15±2.18 (26)	39.78±2.40 (26)
1200	Peak Amp.	833.16±134.97 (29)	472.24±81.56 (29)	374.07±58.57 (29)	348.45±46.66 (29)	356.32±48.76 (29)
	Latency	39.76±1.47 (29)	40.35±1.70 (29)	38.62±1.634(29)	37.86±1.99 (29)	36.52±1.61 (29)

[a] Data were obtained from pages 297 and 299; 10 trials/block.
() The number of animals examined
* Significantly different from controls at $p<0.05$

d. <u>Learning and memory testing</u>: The active avoidance testing data are presented in Tables 12a and 12b below. There were no treatment-related effects observed after reviewing the results of the percent avoidance trial. There were no day-by-treatment interactions for males; however, females exhibited a significant linear day-by-treatment interaction effect at 200 mg/kg/day, as evidenced by significant increases observed in this dose group over time. Because this effect was observed only at the low dose, it was not considered biologically significant.

With regard to adaptation crossing, 700-mg/kg/day males exhibited a significant increase in the number of crossings during the second session on PND 61. This finding was not considered biologically significant because it occurred only on one day and was observed in only one dose group. There were no day-by-treatment effects observed for either sex. Intertrial interval crossings also showed no treatment-related changes between treatment groups and the control and no day-by-treatment effects.

TABLE 12a. Avoidance testing results for F_1 male rats [a]

Dose (mg/kg/day)	Parameter	PND 60	PND 61	PND 62	PND 63	PND 64
Percent avoidances and escapes on PND 60-64						
0	% Avoidance	34.26±6.59226 (27)	25.00±5.74258 (27)	32.41±7.00717 (27)	38.70±7.55 (27)	41.30±8.16319 (27)
	% Escape	94.56±2.13728 (24)	91.56±4.36426 (26)	92.16±5.01910 (24)	95.80±3.08 (25)	98.64±1.36364 (22)
200	% Avoidance	39.51±6.68316 (27)	37.41±6.45150 (27)	41.11±7.01714 (27)	40.56±7.325 (27)	47.41±7.48394 (27)
	% Escape	90.26±5.25825 (23)	89.48±4.94292 (25)	94.19±3.80357 (26)	100.00±0.71 (23)	97.85±2.15311 (22)
700	% Avoidance	53.72±7.10321 (26)	40.00±7.37981 (26)	39.81±7.56346 (26)	43.08±7.52 (26)	53.08±8.36695 (26)
	% Escape	94.55±3.56505 (21)	97.17±2.14478 (21)	99.75±0.25253 (22)	99.54±0.31367 (22)	100.00±0.00000 (20)
1200	% Avoidance	43.27±6.97558 (28)	47.32±7.09666 (28)	42.68±6.72338 (28)	41.07±7.16102 (28)	48.39±7.74534 (28)
	% Escape	89.38±4.77732 (24)	92.99±4.41929 (24)	96.06±2.72656 (24)	100.00±0.00000 (25)	100.00±0.00000 (22)
Adaptation and intertrial crossings on PND 60-64						
0	Adaptation Crossing	11.85±1.03734 (27)	7.15±0.96624 (27)	4.93±0.93359 (27)	6.89±1.20225 (27)	5.30±0.98249 (27)
	Intertrial Crossing	0.22±0.042126 (27)	0.074±.0.022067 (27)	0.16±0.53532 (27)	0.11±0.036844 (27)	0.12±0.031707 (27)
200	Adaptation Crossing	12.59±0.95818 (27)	7.15±0.98378 (27)	6.78±1.03958 (27)	5.74±0.91145 (27)	6.89±1.24417 (27)
	Intertrial Crossing	0.23±0.043514 (27)	0.14±0.038056 (27)	0.13±0.038038 (27)	0.17±0.053185 (27)	0.12±0.034100 (27)
700	Adaptation Crossing	13.96±0.80667 (26)	10.65±1.13807 (26)*	8.38±1.22923 (26)	8.73±1.22607 (26)	7.54±1.04253 (26)
	Intertrial Crossing	0.39±0.075923 (26)	0.20±0.056835 (26)	0.12±0.040216 (26)	0.24±0.063991 (26)	0.19±0.052381 (26)
1200	Adaptation Crossing	12.32±0.97868 (28)	8.25±0.98215 (28)	8.07±1.27130 (28)	7.79±1.14129 (28)	7.61±1.35140 (28)
	Intertrial Crossing	0.30±0.056187 (28)	0.16±0.042729 (28)	0.19±0.048309 (28)	0.24±0.058439 (28)	0.21±0.061821 (28)

[a] Data extracted from pages 304-306 of the study report; means and standard error rates are reported.
() The number of animals examined.
* Significantly different from controls at $p<0.05$

TABLE 12b. Avoidance testing results for F_1 female rats [a]

Dose (mg/kg/day)	Parameter	PND 60	PND 61	PND 62	PND 63	PND 64
Percent avoidances and escapes on PND 60-64						
0	% Avoidance	58.90±7.83 (27)	58.70±7.65 (27)	53.89±7.49 (27)	61.85±8.21 (27)	71.67±7.11 (27)
	% Escape	73.77±7.99 (17)	85.11±4.93 (21)	88.67±6.32 (18)	89.16±6.75 (15)	97.42±1.76 (15)
200	% Avoidance	43.89±7.30 (27)	52.04±8.05 (27)	66.67±7.08 (27)	76.30±6.82 (27)	79.44±7.07 (27)
	% Escape	77.87±6.79 (22)	89.31±6.06(18)	88.51±6.39 (18)	100.00±0.00(13)	100.00±0.00 (8)
700	% Avoidance	64.23±7.263 (26)	57.90±7.52 (26)	69.04±7.88 (26)	83.46±5.53 (26)	83.46±5.86 (26)
	% Escape	82.78±6.36 (16)	97.64±1.77 (18)	98.68±1.32 (16)	100.00±0.00 (12)	100.00±0.00 (11)
1200	% Avoidance	60.86±6.97 (29)	55.34±7.44(29)	57.59±6.82 (29)	59.83±7.82 (29)	71.55±7.4 (29)
	% Escape	77.24±8.09(18)	88.07±6.36 (21)	89.48±5.14 (22)	90.62±6.80 (16)	93.33±6.67 * (15)
Adaptation and intertrial crossings on PND 60-64						
0	Adaptation Crossing	13.80±1.04 (27)	12.07±1.06 (27)	10.37±1.32 (27)	10.11±1.2 (27)	11.78±1.34 (27)
	Intertrial Crossing	0.511±0.08 (27)	0.34±0.07 (27)	0.30±0.06 (27)	0.29±0.07 (27)	0.46±0.08 (27)
200	Adaptation Crossing	12.44±1.03 (27)	11.70±1.08 (27)	11.44±1.47 (27)	11.04±1.29 (27)	13.22±1.27 (27)
	Intertrial Crossing	0.33±0.08 (27)	0.28±0.07 (27)	0.345± 0.06 (27)	0.41±0.06 (27)	0.35±0.05 (27)
700	Adaptation Crossing	14.81±0.84 (26)	13.61±1.18 (26)	13.00±0.86 (26)	13.54±1.21 (26)	16.00±1.04 (26)
	Intertrial Crossing	0.43±0.06 (26)	0.29±0.06 (26)	0.38±0.077 (26)	0.47±0.08 (26)	0.42±0.08 (26)
1200	Adaptation Crossing	13.21±1.18 (29)	12.90±1.01 (29)	10.83±1.36 (29)	10.00±1.41 (29)	12.17±1.77 (29)
	Intertrial Crossing	0.55±0.08 (29)	0.36±0.07 (29)	0.32±0.66 (29)	0.35±0.08 (29)	0.38±0.09 (29)

[a] Data extracted from pages 303 and 305-306 of the study report; means and standard error rates are reported.
() The number of animals examined.
* Significantly different from controls at $p<0.05$

5. Postmortem results:

a. **Brain weights:** Mean brain weight data are presented in Table 13 below. There were no treatment-related changes in terminal body weight, brain weight, or relative brain weight observed at any of the dose levels at either PND 22 or 68. According to the study authors, there was a significant trend of decreasing cerebellum weight (both absolute and relative to total brains weight) among female pups on PND 22. Cerebellum weight was extremely small, however, and no treated group was significantly different from the control. Consequently, the trend was considered spurious, especially since a similar pattern was not observed on PND 68.

TABLE 13. Mean (±standard error rates) brain weight data for pups [a]

Parameter	Dose (mg/kg/day)			
	0	200	700	1200
Males				
PND 22				
Terminal body weight (g)	58.15±1.36 (24)	59.85±1.36 (20)	60.53±0.85 (24)	58.16±1.21 (23)
Brain weight (g)	1.61±0.02 (24)	1.60±0.02 (20)	1.61±0.01 (24)	1.59±0.01 (23)
Brain-to-body weight ratio (g/kg)	2.79±0.06 (24)	2.69±0.05 (20)	2.68±0.04 (24)	2.76±0.05 (23)
PND 68				
Terminal body weight (g)	449.20±7.63 (24)	456.55±6.63 (25)	447.63±4.86 (23)	433.03±6.40 (25)
Brain weight (g)	2.17±0.02 (24)	2.17±0.02 (25)	2.17±0.02 (23)	2.16±0.02 (25)
Brain-to-body weight ratio	0.48±0.01 (24)	0.48±0.01(25)	0.49±0.01 (23)	0.50±0.01 (25)
Females				
PND 22				
Terminal body weight (g)	55.70±1.47 (22)	56.35±1.45 (20)	55.27±1.26 (21)	56.01±1.07 (25)
Brain weight (g)	1.55±0.01 (22)	1.53±0.02 (20)	1.53±0.02 (21)	1.56±0.02 (25)
Brain-to-body weight ratio	2.82±0.06 (22)	2.75±0.06 (20)	2.78±0.06 (21)	2.80±0.05 (25)
PND 68				
Terminal body weight (g)	260.38±4.37 (24)	268.26±4.52 (24)	267.73±6.27 (23)	263.14±4.98 (26)
Brain weight (g)	2.03±0.02 (24)	2.02±0.02 (24)	2.01±0.02 (23)	2.01±0.02 (26)
Brain-to-body weight ratio	0.78±0.01 (24)	0.76±0.02 (24)	0.76±0.02 (23)	0.77±0.01 (26)

[a] Data obtained from page 32 and in the study report.
() The number of animals examined.

b. **Neuropathology:**

1. **Macroscopic examination:** There were no treatment-related macroscopic observations reported at any dose level.

2. **Microscopic examination:** There were no treatment-related microscopic findings. On PND 22, one 1200-mg/kg/day female exhibited remnants of the cerebellar external germinal layer still in place below the meninges. The cerebellar external germinal layer migrates to the Purkinje cell layer to form the internal granular layer in adults. This migration generally occurs during the second week after birth and is essentially completed by the fourth week of postnatal life. Consequently, this finding was not considered treatment-related because the finding was within the normal range

of development and the internal granular cell layer in this animal was intact. Morphometric evaluations were not conducted.

III. DISCUSSION and CONCLUSIONS:

A. CONCLUSIONS: Several study deficiencies are noted below. **The maternal NOAEL is 700 mg/kg/day; the maternal LOAEL is 1200 mg/kg/day based on the one mortality. The NOAEL for developmental neurotoxicity is ≥1200 mg/kg/day. The LOAEL could not be calculated.**

In addition, the following study deficiencies are noted:

- The following observations/measurements in dams were not recorded: ranking of the degree of lacrimation and salivation, with a range of severity scores from none to severe; presence of exophthalmus; and degree of palpebral closure.
- Mean litter size was not provided for PND 11, 17, and 21.
- Live birth index, viability index, and lactation index were not calculated.
- The study report did not provide the number born, the number born live, or the number born dead. The individual pup body weight data on PND 0 for each dose group was used to count the number of males and females, assuming that these pups were alive.
- A functional observational battery was not conducted for pups.
- According to the guidelines, neuropathological examination should be conducted PND 11 and at the termination of the study. For this study, brain weights were recorded on PND 22 and at termination. Microscopic evaluations also were conducted on PND 22 and at termination.
- According to the guidelines, neuropathological examination should be conducted for 6 animals per sex per dose at both interim and terminal sacrifice. For this study, only three animals per sex per dose were assessed at each sacrifice.
- A morphometric analysis was not performed on PND 11 or at the termination of the study to assess the structural development of the brain.

D. STUDY CLASSIFICATION: This study is classified **ACCEPTABLE / Not Guideline** and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, OECD 426).